Modified Method for Rapid Determination of Individual Mononucleotides

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SUMMARY

An ion-exchange chromatography method for separation and quantitative analysis of 5′-mononucleotides is described. Five mononucleotides commonly found in foods can be analyzed in half the time required by similar procedures and four samples can be analyzed daily by using two columns and one fraction collector.

During a series of studies on heat-induced changes in perchloric-acid-extractable mononucleotides in beef, lamb, and pork muscle it was found possible to modify the ion-exchange method of Lento *et al.* (1964) to give separations of cytidine monophosphate (CMP), adenosine monophosphate (AMP), uridine monophosphate (UMP), inosine monophosphate (IMP), and guanosine monophosphate (GMP) in 4–5 hr rather than over the 12 hr required in the original method. The modifications consisted of changing the amount of distilled water in the mixing chamber leading to the column from 250 ml to 125 ml and increasing flow rate from 1 ml per min to 2.5 ml per min. Ten-ml fractions were collected with an Isco fraction collector, Model A, and the absorbance of the effluent was monitored with an Isco ultraviolet flow densitometer, Model U, at 254 mμ. A Beckman Accu-Flo chromatographic pump was used to maintain the desired flow rate. It was also found that two columns could be eluted simultaneously using the same fraction collector with an additional pump for the second column. When this was done, the end points of the peaks from the second column were checked with a Beckman spectrophotometer, Model DU, at 260 mμ, using the recorder strip chart from the effluent of the first column as a reference. Columns consisted of 10 mm ID × 400-mm glass tubing constricted at one end and plugged with glass wool and glass beads. The ion-exchange resin used was Bio Rad Laboratories analytical-grade anion-exchange resin AG 1X8, 200–400-mesh. It was converted to the hydroxyl form by stirring intermittently with 3 bed volumns 1*N* sodium hydroxide for 2 hr. Excess base was removed by filtering in a Büchner funnel and the resin washed with 1 L distilled water. Further washing was accomplished by suspending the resin in 1 L distilled water, stirring and filtering. The latter procedure was repeated 3 times and the moist resin converted to the formate form by treatment for 2 hrs with 2 bed volumes of 6*N* formic acid. Excess acid was removed by filtration and the washing procedure repeated. The resin was suspended in an equal volume of distilled water and dissolved air removed by

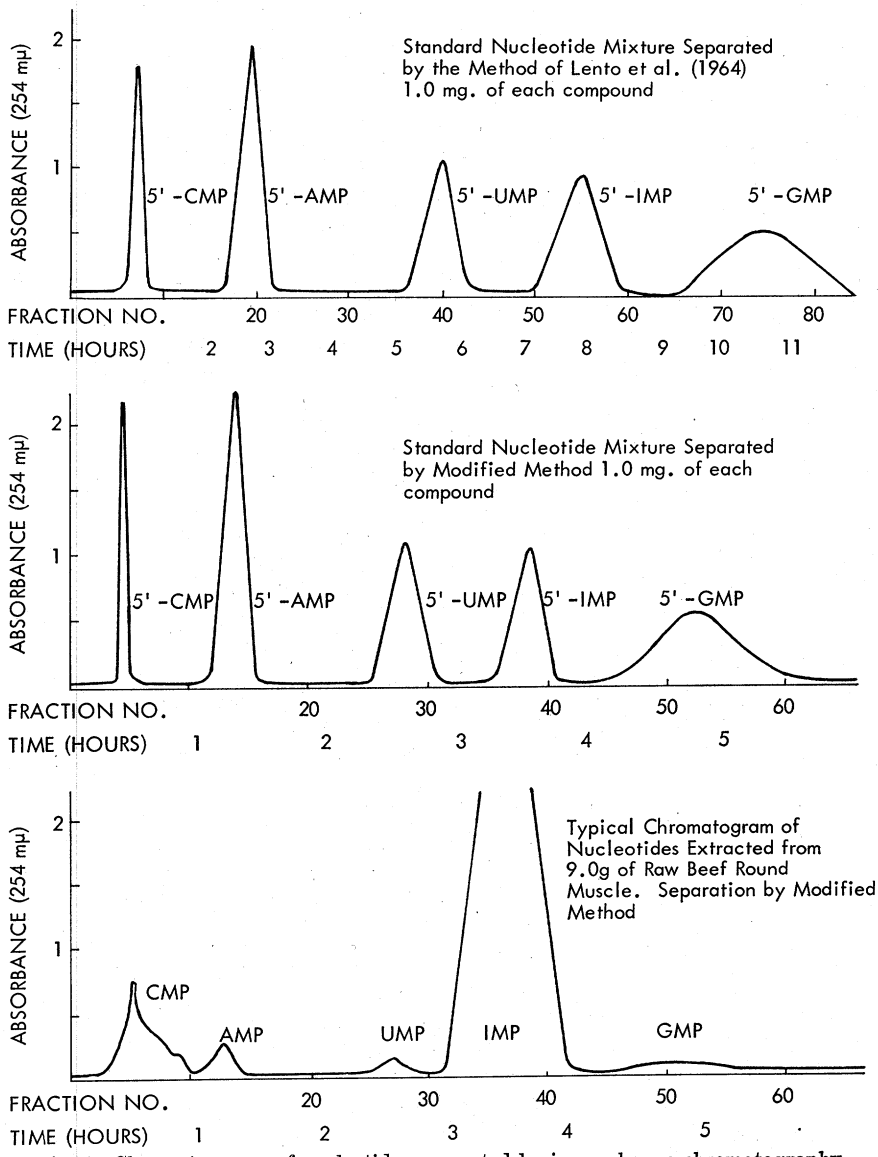

Fig. 1. Chromatograms of nucleotides separated by ion-exchange chromatography.

aspiration. Slurry resulting from rapid shaking was poured into glass chromatographic tubes to form beds 160 mm deep and uniform packing obtained by applying about 2 meters water pressure for 20 min. Finally, a piece of filter paper 10 mm in diameter was tamped onto the top of the column to prevent resin disturbance during sample application. Fig. 1 shows chromatograms obtained by the unmodified method, modified method, and a typical chromatogram by the modified method of perchloric-acid-extractable nucleotides of raw beef. Since only absorbance at 254 mμ was used to measure nucleotide content during their separation, no differentiation was made between the 2′, 3′, and 5′ isomers of the nucleotides. This could be readily accomplished with the periodate method described by Lento *et al.* (1964). Calibration of the columns and quantitation of the nucleotides were carried out as in the original method. The modified method allows convenient separation of four samples per day with one fraction collector.

REFERENCES

Lento, H. G., J. A. Ford, and A. E. Denton. 1964. A method for determining 5′-nucleotides. *J. Food Sci.* **29**, 435.

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